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ORIGINAL RESEARCH

Prognostic Value of the *TP53* Mutation Location in Metastatic Breast Cancer as Detected by Next-Generation Sequencing

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Purpose: The status of *TP53* mutations was measured in cell-free DNA from patients with metastatic breast cancer (MBC) to investigate disease characteristics and the prognostic role of different locations of the *TP53* mutation site.

Patients and Methods: Blood samples were taken from a total of 187 patients diagnosed with MBC who were treated at the Department of Breast Oncology, Peking University Cancer Hospital between January 2013 and March 2020. Next-generation sequencing was used to investigate the *TP53* mutation spectra of circulating free DNA in these blood samples.

Results: Among the 187 MBC patients, *TP53*-mutated patients had a significantly shorter median disease-free survival (DFS) and overall survival (OS) compared with *TP53* wild-type patients (P=0.001 and P=0.006, respectively). Additionally, in hormone receptor positive/HER2 negative (HR+/HER2-) and triple negative (TNBC) cohorts, *TP53*-mutated patients had a significantly shorter median DFS than *TP53* wild-type patients (P=0.038 and P=0.023, respectively). The 79 patients with *TP53* mutations carried 87 somatic *TP53* mutations, of which most (77.0%) mapped to the DNA-binding domain (DBD) of the protein encoded by *TP53* exons 5–8. In patients with *TP53* mutations, those occurring in the non-DBD had a significantly shorter median DFS and OS than *TP53* wild type (P<0.001 and P=0.001, respectively). Additionally, patients with non-missense mutations in the DBD had a significantly shorter median DFS and OS than *TP53* wild-type patients (P=0.001 and P<0.001, respectively). *TP53*-mutated patients had a significantly shorter DFS than *TP53* wild-type patients in the adjuvant endocrine therapy sensitive group (P=0.008), but differences in the endocrine therapy resistant group were not significant.

Conclusion: *TP53*-mutated MBC patients had a significantly worse outcome than *TP53* wild-type patients especially those in HR+/HER2– and TNBC cohorts. Of *TP53*-mutated patients, those with non-missense mutations in the DBD had worse breast cancer-related survival. *TP53* mutations were also associated with endocrine resistance.

Keywords: advanced breast cancer, *TP53* mutation, NGS, adjuvant endocrine therapy, DNA-binding domain

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Introduction

As a tumor suppressor and DNA binding transcription factor, *TP53* is actively involved in the regulation of the cell cycle, apoptosis, and genomic stability.[1,2] *TP53* is one of the most frequently mutated genes in human cancers, including breast cancer,[3] and numerous studies have reported it as a biomarker for predicting an aggressive and metastatic phenotype in breast cancer.[4–7] Most of these studies

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used first-generation sequencing or automated DNA extraction from formalin-fixed and paraffin-embedded tissue (FFPE). However, real-time (RT)-PCR results and first-generation sequencing could not be used to detect all *TP53* mutations to further investigate *TP53* status more accurately and reliably.

TP53 is located on chromosome 17p13.1 and contains 11 exons and 10 introns. Most *TP53* mutations map to exons 5–8, which encodes the DNA binding domain (DBD), and most are missense mutations.[8–10] Hotspot codons 175, 213, 245, 248, 273, and 282 account for at least 2% of all mutations within the DBD.[2] Patients with acute myeloid leukemia carrying *TP53* mutations in the DBD had a worse prognosis than those with wild-type *TP53*.[11] Furthermore, another clinical trial showed that truncating mutations in the DBD had a significant independent prognostic value in breast cancer, being associated with increased recurrence compared with patients with non-modified p53 proteins.[12]

Early studies using first-generation sequencing or automated DNA extraction from FFPE found that *TP53* mutations were associated with poor prognosis in hormone receptor-positive (HR+) breast cancer patients.[13–15] Moreover, in an HR+ cohort, *TP53* signaling was enriched in resistant tumors (38% in the aromatase inhibitor-resistant group vs 17% in the sensitive group) such that HR+ tumors with *TP53* mutations were mostly aromatase inhibitor-resistant.[5] No significant result was obtained from human epidermal growth factor receptor 2 positive (HER2+) or triple-negative breast cancer (TNBC) cohorts.[16] Other studies found that *TP53* mutations were associated with tumor recurrence and apoptosis, which were more common in HER2-positive and TNBC cohorts.[17,18]

While the significance of *TP53* mutations has been shown by RT-PCR and first-generation sequencing, most clinical laboratories do not use next-generation sequencing (NGS) to determine the p53 mutational status because of high costs and complex interpretation. Therefore, it is difficult to understand the clinical applications of *TP53*.[19] In the present study, we collected peripheral blood samples from Chinese patients with freshly diagnosed metastatic breast cancer (MBC) and examined the whole exons and introns of *TP53* by NGS to further investigate the relationship between *TP53* mutations, prognosis, and therapy.

Materials and Methods

Patients

From January 2013 to March 2020, patients past first-line treatment and those for whom blood samples were not available were excluded, leaving a total of 194 at the stage of first-line treatment at the Department of Breast Oncology, Peking University Cancer Hospital. Of these, 187 consented with enrollment and had complete clinicopathological information (Figure 1).

We defined estrogen receptor (ER), progesterone receptor (PR), and HER2 status according to recommended guidelines,[20,21] which identified three subtypes: the HER2+ cohort, HR+/HER2- cohort, and TNBC cohort.

HR+/HER2- patients who accepted adjuvant endocrine therapy were divided into two groups: endocrine-resistant patients were defined as patients relapsing during adjuvant endocrine therapy, or <12 months after its completion. Endocrine-sensitive patients were defined as patients relapsing ≥12 months after completing adjuvant endocrine therapy in the early breast cancer stage.[22]

Samples

Peripheral blood samples before first-line therapy were collected in EDTA Vacutainer tubes and centrifuged at 2000 g for 10 min at 4°C. The supernatant was then removed, and each sample of 3 mL plasma was stored at –80°C.

Circulating Free DNA Extraction

Circulating free (cf)DNA was extracted using a QIAamp Circulating Nucleic Acid Kit (QIAamp, Venlo, the Netherlands) from EDTA and citrate anticoagulant plasma. The average volume of plasma used for extraction was 2.6 mL (range, 0.7–3.9 mL). The quantity and quality of the purified cfDNA were checked using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). For samples with severe genomic contamination from peripheral blood cells, size selection was performed to remove large genomic fragments with AMPure XP beads (Beckman Coulter, Brea, CA, USA). Samples with a total yield <5 ng were considered inadequate for NGS and were removed from any further sequencing methods.

Library Preparation, Capture, and Sequencing

cfDNA was end-repaired before the dA-tailing process, and then ligated with proprietary UMI adapters. The library yield was measured after PCR amplification using a Qubit and Bioanalyzer 2100. Samples yielding >700 ng proceeded to the hybridization step. Library capture was conducted using biotin-labeled DNA probes (Thermo Fisher Scientific). In

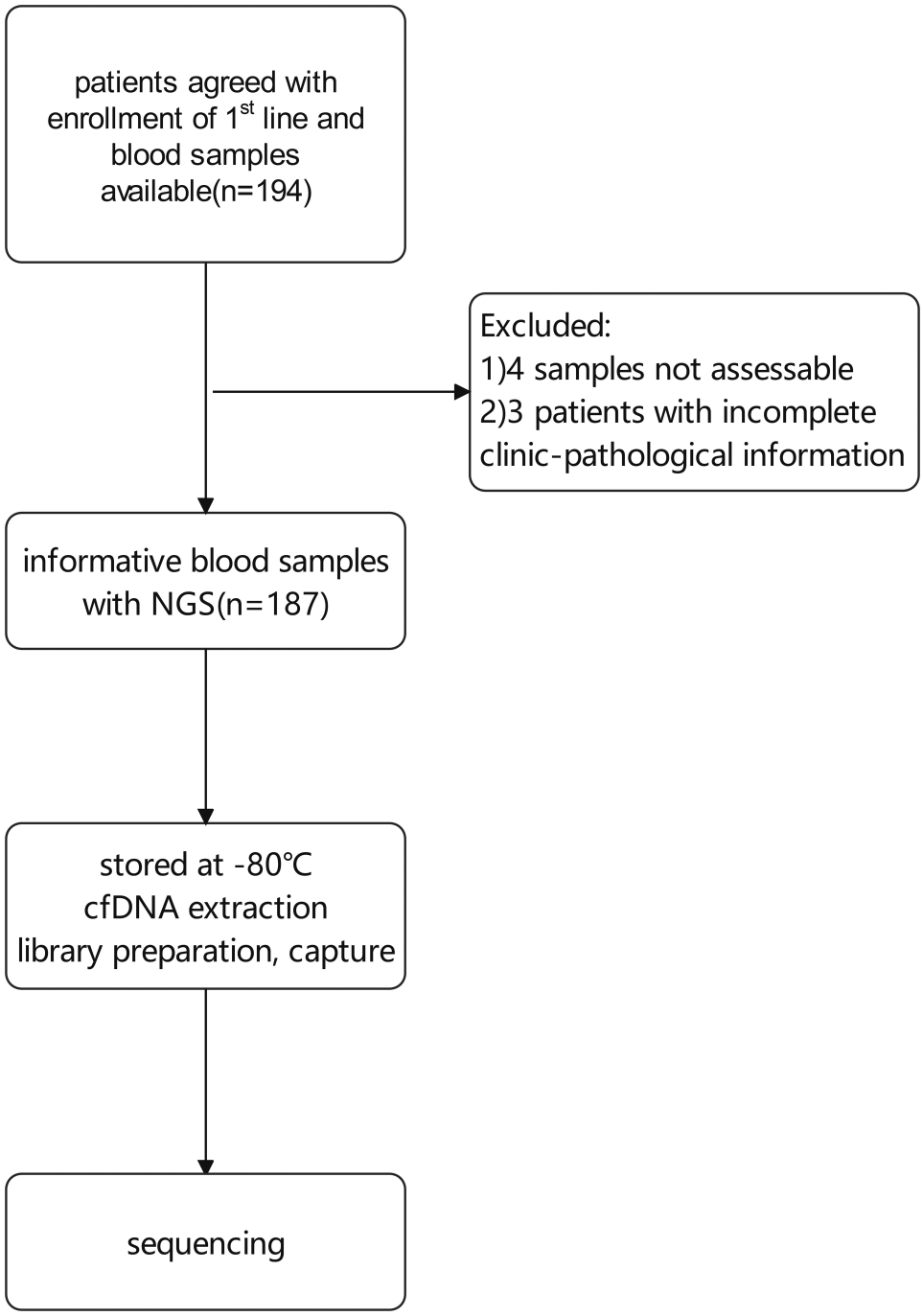

Figure 1 Flowchart of patient inclusion.

brief, the library was hybridized using PredicineCARE panel (Huidu Shanghai Medical Sciences, Inc.) overnight and captured on Dynabeads M-270 Streptavidin (Thermo Fisher Scientific).[23,24] Unbound fragments were washed away, and the enriched fragments were amplified via PCR. For library preparation, the purified product was checked using Bioanalyzer 2100 and loaded into the HiSeq X Ten system (Illumina, San Diego, CA, USA) for NGS with paired-end 150 bp sequencing kits.

Analyses of NGS Data Generated from cfDNA

Consensus binary alignment map (BAM) files were derived by merging paired-end reads that originated from

the same molecules (based on mapping location and unique molecular identifiers) as single-strand fragments. Single-strand fragments from the same double-strand DNA molecules were merged to be double-stranded for suppressing sequencing and PCR errors during this process. NGS quality-checking was performed by examining the percentage of targeted regions with >1500x unique consensus coverage. Samples with <80% regions having >1500x unique coverage were deemed to be QC failed and excluded. Candidate variants, consisting of point mutations, small insertions and deletions, were identified using Huidu proprietary bioinformatics pipeline. Candidate variants with low base quality, mapping scores, and other quality metrics were filtered. Candidate variants in repeat regions were also excluded.

A variant identified in cfDNA was considered to be a candidate somatic mutation-based if all of the following pre-defined criteria were present. These criteria were 1) the presence of at least 4 distinct paired reads in the mutation in the plasma; 2) the number of distinct paired reads containing a particular mutation in the plasma is at least 0.1% of the total distinct read pairs (if the nucleotide change and amino acid change are identical to an alteration observed in ≥20 cancer cases reported in the COSMIC database or previously reported as a cancer hotspot [http://www.cancerhotspots.org]) or the number of distinct paired reads containing a particular mutation in the plasma was at least 0.25% of the total distinct read pairs (if the nucleotide change and amino acid change are not a frequent alteration in COSMIC database or reported as a cancer hotspot previously); 3) the variant is not present in public databases of common germline variants, including 1000 genomes, ExAC, gnomAD, and KAVIAR, with population allele frequency >0.5%; 4) the variant is not present in matched PBMC samples (unpublished data, manuscript in preparation).

Candidate somatic mutations were further filtered based on gene annotation to identify those occurring in protein-coding regions. Intronic and silent changes were excluded, and mutations resulting in missense mutations, nonsense mutations, frameshifts, or splice site alterations were retained. Mutations annotated as benign or likely benign in ClinVar database were also filtered.

Evaluation

Clinical outcome was evaluated as disease-free survival (DFS) and overall survival (OS). Disease-free survival (DFS) was defined as the interval between surgery and time of recurrence for relapsed patients so that patients with stage IV were not included. OS was defined as the time from diagnosis to the date of death or last follow-up. According to Response Evaluation Criteria in Solid Tumors version 1.1 guidelines,[25] we evaluated the response assessment by a computed tomography scan or magnetic resonance imaging every 6–12 weeks or as the patient's condition deteriorated.

Statistical Analysis

SPSS software version 20 was used to analyze the *TP53* status and categorical patient characteristics. DFS and OS were estimated by the Kaplan–Meier method and comparisons between groups were conducted by the log rank test. P values <0.05 were considered significant. For multivariable analysis, Cox proportional hazards method was used to evaluate clinical outcome. The association between the *TP53* status and clinical characteristics was examined using the Chi-square test.

Results

Patient Characteristics

Of 187 patients, 79 carried *TP53* mutations and 108 had wild-type *TP53*. Detailed baseline clinical information of all patients is shown in Table 1. The median age in the *TP53* mutated group was 48 years (range: 27–69 years old) versus 46 years of age in the *TP53* wild-type group (range: 26–80 years old) (P = 0.702). We also found that 73.4% (58/79) of *TP53*-mutated patients and 86.1% (93/108) of *TP53* wild-type patients were HER2 negative (P=0.030).

In univariate analysis of DFS (Table 2), HER2 status (P=0.024) and HR status (P=0.000) were significant predictors in *TP53* wild-type patients and *TP53*-mutated patients, respectively, and Ki67 status was also a significant predictor for *TP53* wild-type patients (P=0.001) and *TP53*-mutated patients (P=0.022). After multivariable analysis of DFS (Table 2), Ki67 status (P=0.003) and HR status (P=0.000) in *TP53* mutated group remained significant predictors and patients with stage III had a higher risk of relapse after surgery than stage I–II (p=0.030) in *TP53* wild-type cohort.

Characteristics of TP53-Mutated Patients

A total of 87 somatic *TP53* mutations were identified in the 79 *TP53*-mutated patients. Sixty-seven of these (77.0%) were located in exons 5–8, which span the DBD of the protein (Supplementary Table S1). Codons 175, 220, and 248 within the DBD were the locations of 4.6% of all mutations, respectively, which were all missense

Table 1 Baseline Clinical Characteristics of *TP53* Wild-Type and -Mutated Metastatic Breast Cancer Patients (n=187)

Characteristics	*TP53* Status		p value
	Wild-Type (n=108)	Mutated (n=79)	
Age at diagnosis (years)			
Median age(range)	46(26–80)	48(27–69)	
≤50	64(59.3%)	49(62.0%)	0.702
>50	44(40.7%)	30(38.0%)	
Family history			
Breast/ovarian cancer	7(6.5%)	3(3.8%)	0.688
Other cancers	17(15.7%)	14(17.7%)	
No	84(77.8%)	62(78.5%)	
Stage at diagnosis			
I~II	52(48.1%)	29(36.7%)	0.136
III	22(20.4%)	22(27.9%)	
IV	18(16.7%)	20(25.3%)	
Unknow	16(14.8%)	8(10.1%)	
Grade			
I–II	53(49.1%)	41(51.9%)	0.563
III	13(12.0%)	13(16.5%)	
Unknow	42(38.9%)	25(31.6%)	
Ki67			
1~20%	35(32.4%)	35(44.3%)	0.245
>20%	55(50.9%)	38(48.1%)	
Unknow	18(16.7%)	6(7.6%)	
HR status			
Positive	85(78.7%)	53(67.1%)	0.074
Negative	23(21.3%)	26(32.9%)	
HER2 status			
Positive	15(13.9%)	21(26.6%)	0.030
Negative	93(86.1%)	58(73.4%)	
Lymph node status			
Positive	65(60.2%)	44(55.7%)	0.554
Negative	42(38.9%)	34(43.0%)	
Unknow	1(0.9%)	1(1.3%)	
Disease involvement			
Visceral	64(59.3%)	46(58.2%)	0.848
Non-visceral	42(38.9%)	32(40.5%)	
Unknow	2(1.9%)	1(1.3%)	
Adjuvant endocrine therapy			
Tamoxifen/toremifene	41(37.9%)	23(29.1%)	0.238
Aromatase inhibitor	14(13.0%)	10(12.6%)	
Tamoxifen+ aromatase inhibitor	3(2.8%)	3(3.8%)	
Others	1(0.9%)	1(1.3%)	
No	49(45.4%)	42(53.2%)	

(*Continued*)

Table 1 (Continued).

Characteristics	*TP53* Status		p value
	Wild-Type (n=108)	Mutated (n=79)	
Adjuvant chemotherapy			
Paclitaxel	9(8.4%)	12(15.2%)	0.134
Anthracycline	23(21.3%)	7(8.9%)	
Paclitaxel+ anthracycline	36(33.3%)	25(31.6%)	
Others	4(3.7%)	4(5.1%)	
No	36(33.3%)	31(39.2%)	
Adjuvant targeted therapy			
Yes	2(1.8%)	4(5.1%)	0.223
No	99(91.7%)	73(92.4%)	
Unknow	7(6.5%)	2(2.5%)	

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; DFS, disease free survival.

mutations (Figure 2). Of the 87 mutations, there were 46 missense mutations (43 was in DBD, 1 was in TD, 1 was in TAD, and 1 was outside the p53 protein domain) and 41 non-missense mutations (18 nonsense mutations, 3 splicing mutations, 16 frameshift mutations, 4 in-frame mutations).

TP53 Status in Different Subtypes

We found that the median DFS of *TP53*-mutated patients was significantly shorter at 33.0 months (95% confidence interval [CI]=21.4–44.6) than that of *TP53* wild-type patients at 51.0 months (95% CI=39.1–60.9) (hazard ratio=1.89, 95% CI=1.31–2.71, P=0.001) (Figure 3A). Similarly, the median OS of *TP53*-mutated patients was significantly shorter at 67.0 months (95% CI=44.4–89.6) than that of *TP53* wild-type patients at 140.0 months (95% CI=119.5–160.5) (hazard ratio=1.99, 95% CI=1.21–3.26, P=0.006) (Figure 3B).

In the HER2+ cohort (n=36, 21 of whom were *TP53*-mutated patients), there was no significant difference regarding *TP53* status with respect to DFS (34.0 vs 21.0 months, P=0.822) (Figure 3C) or OS (91.0 vs 65.0 months, P=0.080) (Figure 3D).

In the HR+/HER2- cohort (n=113, 40 of whom were *TP53*-mutated patients), the median DFS of *TP53* mutated patients of 44.0 months (95% CI=35.9–52.1) was significantly shorter than the 58.0 months (95% CI=46.2–69.8) of *TP53* wild-type patients (hazard ratio=1.57, 95% CI=0.97–2.54, P=0.038) (Figure 3E). No significant difference was observed for OS (P=0.606) (Figure 3F).

In the TNBC cohort (n=38, 18 of whom were *TP53*-mutated patients), the median DFS of *TP53*-mutated patients of 16.0 months (95% CI=7.8–24.2) was significantly shorter than the 26.0 months (95% CI=16.6–35.4) of *TP53* wild-type patients (hazard ratio=2.17, 95% CI=0.96–4.90, P=0.023) (Figure 3G). There was no significant difference regarding *TP53* status with respect to OS (137.0 vs 54.0 months, P=0.117) (Figure 3H).

DBD Missense Mutations Were Associated with Improved Survival

We next classified the 187 patients into three groups by mutation domain: *TP53* mutations in the DBD, *TP53* mutations in the non-DBD, and *TP53* wild-type groups. The median DFS for these patients was 36.6 (95% CI=25.3–42.7), 22 (95% CI=16.1–25.9), and 51 (95% CI=39.1–60.9) months, respectively, while the median OS was 80 (95% CI=46.3–113.7), 51 (95% CI=41.2–60.8), and 140 (95% CI=119.5–160.5) months, respectively.

TP53 wild-type patients had a significantly better clinical outcome than those with *TP53* mutations in the DBD with respect to DFS (P=0.008, Figure 4A) and OS (P=0.003, Figure 4B). Similarly, *TP53* wild-type patients had a significantly better clinical outcome than those with *TP53* mutations in the non-DBD with respect to DFS (P<0.001, Figure 4A) and OS (P=0.001, Figure 4B). There were no significant differences in DFS or OS between patients with *TP53* mutations in the DBD compared with those in the non-DBD.

And then, we divided patients into three groups: *TP53* wild-type group; protein stable mutations group (non-truncating and non-frame altering mutations outside of the p53 tetramerization domain); protein non-stable

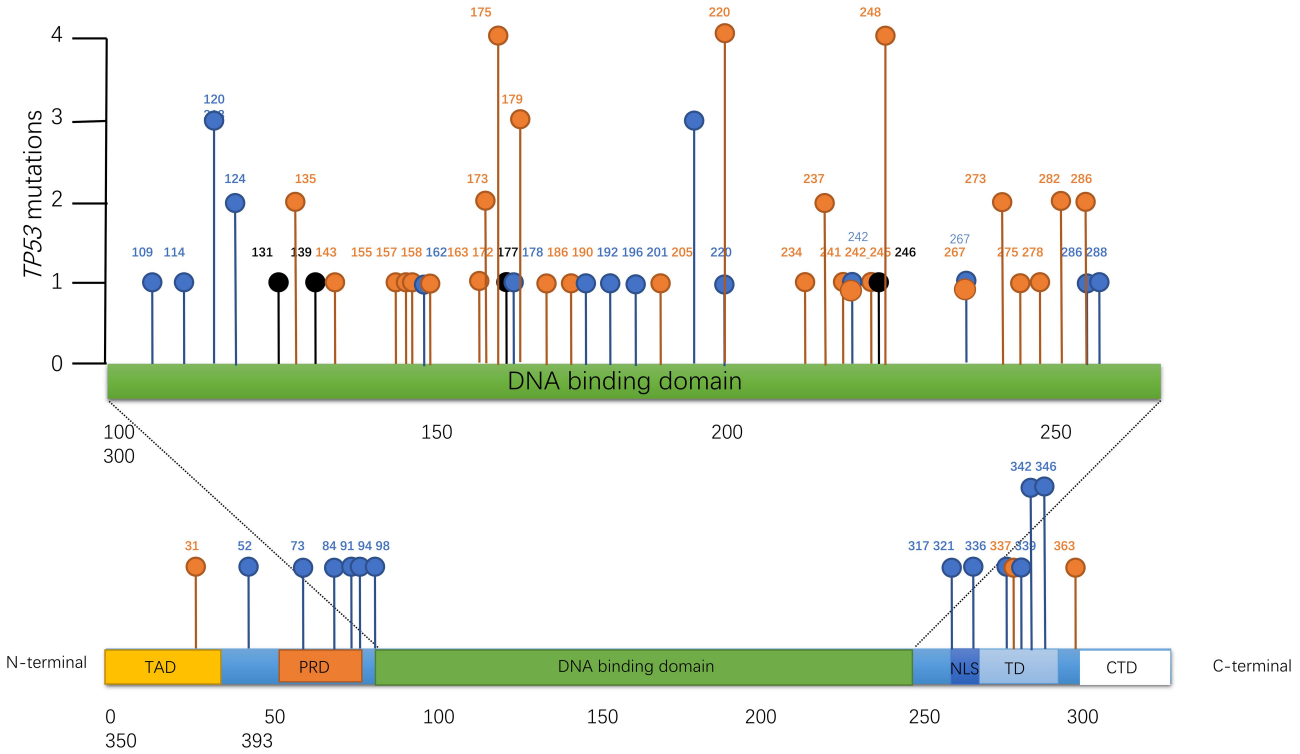

Figure 2 The mutational spectra of TP53 in TP53-mutated patients. (●) Missense (●) Truncating (●) Inframe.
Notes: According to http://www.cbioportal.org, truncating mutations included nonsense mutations, frameshift mutations and splicing mutations.

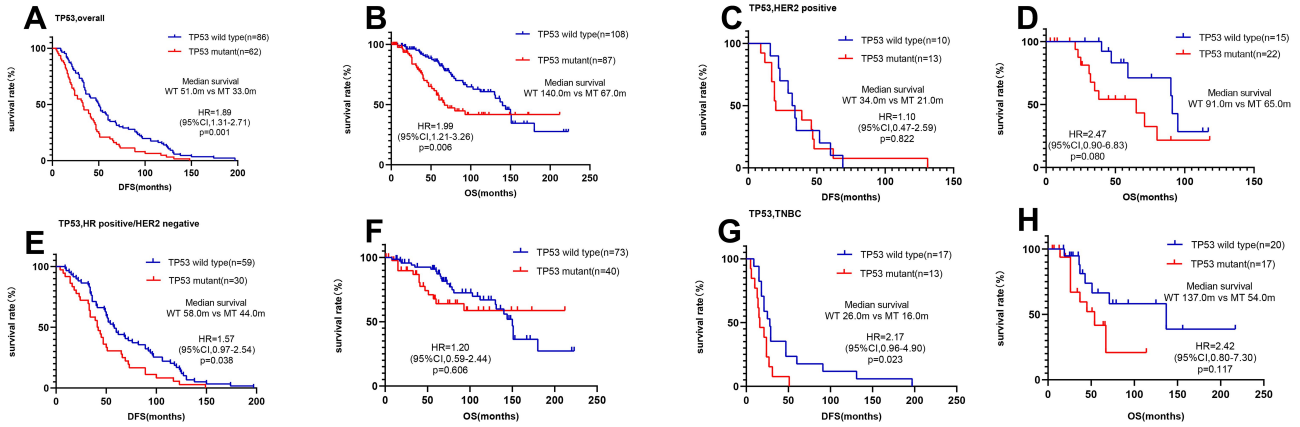

Figure 3 Survival analyses by Kaplan–Meier according to TP53 status in MBC patients. (**A** and **B**) TP53 wild-type patients had a significantly better clinical outcome than TP53-mutated patients. (**C** and **D**) there were no significant differences between TP53 wild-type and -mutated patients in the HER2-positive cohort. (**E** and **F**) TP53 wild-type patients had a significantly longer median DFS and OS than TP53-mutated patients in the HR+/HER2– cohort. (**G** and **H**) TP53 wild-type patients had a significantly longer median DFS than TP53-mutated patients in the TNBC cohort.

mutations group (all truncating and frame-altering mutations, and mutations in the tetramerization domain).

Patients with protein non-stable mutations had significantly shorter DFS (21.0 months vs 49.0 months, respectively, hazard ratio=2.82, 95% CI=1.63–4.87, P<0.001, Figure 4C) and OS (57.0 months vs 140.0 months, respectively, hazard ratio=4.05, 95% CI=1.95–8.40, P<0.001, Figure 4D) than *TP53* wild-type patients. Moreover, the median DFS of protein stable mutations was 43.5 months, longer than protein non-stable mutations (hazard ratio=0.54, 95% CI=0.31–0.93, P=0.025, Figure 4C). There were no significant differences in DFS or OS between patients with protein stable mutations and *TP53* wild type.

Furthermore, we wanted to study mutations in DBD so that we classified them into missense (n=43) and non-missense mutations (n=24, including nonsense mutations,

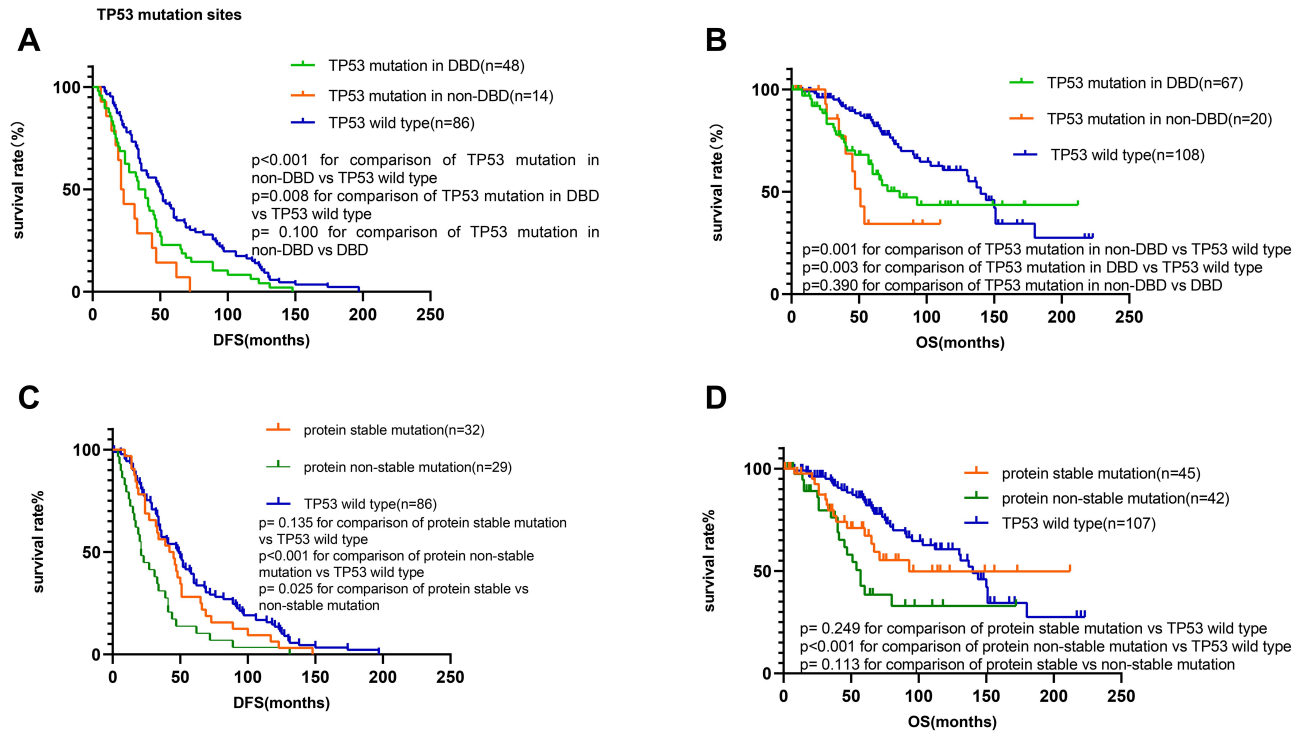

Figure 4 Survival analyses by Kaplan–Meier according to TP53 mutation sites in MBC patients. (**A**) Patients with a mutation in the non-DNA binding domain had a significantly shorter median DFS than TP53 wild-type patients and those with mutations in the DNA-binding domain. (**B**) Patients with a mutation in the non-DNA binding domain had shorter median OS than TP53 wild-type patients and those with mutations in the DNA-binding domain. (**C**) Patients with protein non-stable mutation had shortest median DFS than patients with protein stable mutation and TP53 wild-type patients. (**D**) Patients with protein non-stable mutation had shortest median OS than patients with protein stable mutation and TP53 wild-type patients.

Notes: Protein stable mutations would include non-truncating and non-frame altering mutations outside of the p53 tetramerization domain, and protein non-stable mutations would include all truncating and frame-altering mutations, as well as mutations in the tetramerization domain.

splicing mutations, frameshift mutations and in-frame mutations). Patients with non-missense mutations in the DBD had significantly shorter DFS (20.0 months vs 51.0 months, respectively, hazard ratio=3.26, 95% CI=1.58–6.71, P=0.001, Figure 5A) and OS (57.0 months vs 140.0 months, respectively, hazard ratio=10.45, 95% CI=3.79–28.8, P<0.001, Figure 5B) than *TP53* wild-type patients. Moreover, the median OS of patients with non-missense mutations in the DBD was significantly shorter than those with missense mutations in the DBD (hazard ratio=2.45, 95% CI=1.05–5.09, P=0.015, Figure 5B). There were no significant differences in DFS or OS between patients with missense mutations in the DBD and wild-type *TP53* patients.

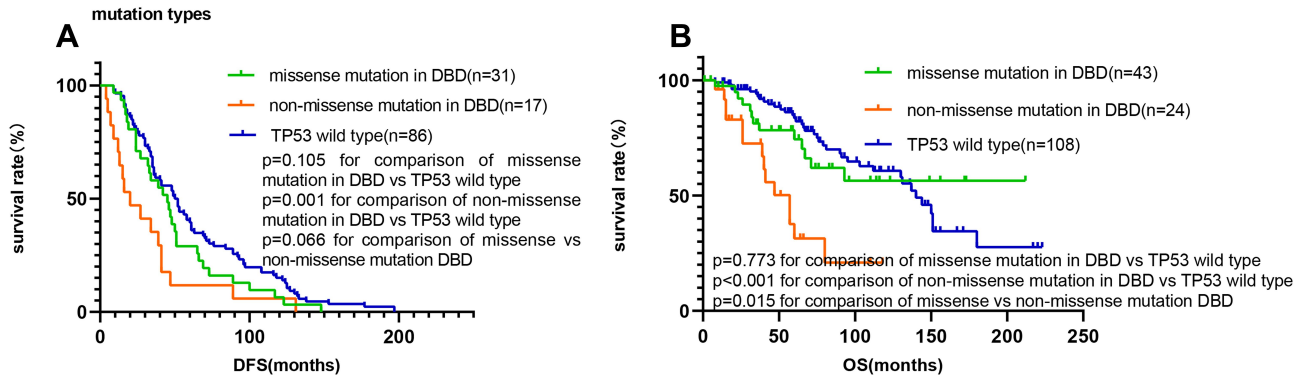

Figure 5 Survival analyses by Kaplan–Meier according to TP53 mutation type in the DNA binding domain. (**A** and **B**) Patients with non-missense mutations in the DNA binding domain had a significantly shorter median DFS and OS than TP53 wild-type patients and those with missense mutations in the DNA binding domain.

Table 2 Univariate and Multivariate Cox Regression Analysis of DFS in *TP53* Wild-Type and -Mutated Patients

	TP53 Wild-Type Group							*TP53* Mutated Group						
	Univariate					Multivariate		Univariate					Multivariate	
	N	%	DFS			HR (95% CI)	p value	N	%	DFS			HR (95% CI)	p value
			mDFS (Range, Month)	HR (95% CI)	p value					mDFS (Range, Month)	HR (95% CI)	p value		
Age at diagnosis (years)														
≤50	64	59.3	51.0(9.0–197.0)	1.00		1.00		49	62.0	28.0(4.0–148.0)	1.00		1.00	
>50	44	40.7	50.0(16.0–197.0)	1.09(0.70–1.70)	0.713	1.22(0.55–2.67)	0.626	30	38.0	40.0(7.0–131.0)	0.77(0.44–1.36)	0.363	0.44(0.20–0.98)	0.044
Family history														
No	84	77.8	52.0(9.0–197.0)	1.00		1.00		62	78.5	32.0(5.0–148.0)	1.00		1.00	
Breast/ovarian cancer	7	6.5	30.0(10.0–124.0)	1.62(0.65–4.05)	0.300	1.20(0.14–10.20)	0.870	3	3.8	23.0(19.0–123.0)	0.75(0.23–2.46)	0.634	3.24(0.41–25.81)	0.267
Other cancers	17	15.7	46.5(9.0–125.0)	1.28(0.69–2.39)	0.435	1.52(0.43–5.32)	0.515	14	17.7	39.0(4.0–73.0)	1.12(0.54–2.32)	0.756	0.84(0.37–1.94)	0.688
Stage at diagnosis														
I–II	52	56.5	49.5(9.0–177.0)	1.00		1.00		29	40.8	21.5(4.0–72.0)	1.00		1.00	
III	22	23.9	36.0(9.0–197.0)	1.11(0.66–1.87)	0.703	2.20(1.08–4.48)	0.030	22	31.0	28.0(5.0–148.0)	0.70(0.39–1.27)	0.241	0.78(0.38–1.58)	0.487
IV	18	19.6	NA	NA	NA	NA	NA	20	28.2	NA	NA	NA	NA	NA
Grade														
I–II	53	80.3	49.0(9.0–177.0)	1.00		1.00		41	75.9	32.0(9.0–148.0)	1.00		1.00	
III	13	19.7	41.0(17.0–197.0)	0.69(0.34–1.40)	0.310	1.04(0.20–5.49)	0.966	13	24.1	21.5(4.0–89.0)	1.52(0.77–3.01)	0.227	0.70(0.12–4.17)	0.699
Ki67														
≤20%	35	38.9	60.5(16.0–177.0)	1.00		1.00		35	47.9	43.0(7.0–148.0)	1.00		1.00	
>20%	55	61.1	34.0(9.0–133.0)	2.40(1.46–3.96)	0.001	1.90(0.99–3.64)	0.054	38	52.1	21.0(4.0–131.0)	1.96(1.10–3.49)	0.022	3.47(1.53–7.89)	0.003
HR status														
Positive	85	78.7	56.0(9.0–197.0)	1.00		1.00		53	67.1	46.0(4.0–148.0)	1.00		1.00	
Negative	23	21.3	26.0(9.0–197.0)	1.58(0.94–2.66)	0.087	2.09(0.97–4.49)	0.059	26	32.9	19.0(5.0–51.0)	4.31(2.24–8.27)	0.000	4.40(1.97–9.84)	0.000
HER2 status														
Positive	15	13.9	34.0(17.0–71.0)	1.00		1.00		21	26.6	21.0(9.0–131.0)	1.00		1.00	
Negative	93	86.1	52.0(9.0–197.0)	0.45(0.23–0.90)	0.024	0.48(0.19–1.20)	0.117	58	73.4	33.0(4.0–148.0)	0.85(0.45–1.59)	0.600	1.18(0.49–2.83)	0.710
Lymph node status														
Negative	42	39.3	51.0(9.0–197.0)	1.00		1.00		34	43.6	31.0(4.0–131.0)	1.00		1.00	
Positive	65	60.7	51.0(14.0–197.0)	1.10(0.71–1.70)	0.663	0.98(0.23–4.22)	0.976	44	56.4	39.0(5.0–148.0)	0.85(0.50–1.44)	0.538	1.00(0.28–3.55)	1.00
Disease involvement														
Non-visceral	42	39.6	40.5(9.0–153.0)	1.00		1.00		32	40.5	29.5(5.0–148.0)	1.00		1.00	
Visceral	64	60.4	52.0(14.0–197.0)	0.70(0.45–1.10)	0.124	1.43(0.68–3.01)	0.343	46	59.5	36.0(4.0–123.0)	0.87(0.49–1.54)	0.640	1.65(0.74–3.69)	0.222

(*Continued*)

Table 2 (Continued).

	TP53 Wild-Type Group							*TP53* Mutated Group						
	Univariate				Multivariate			Univariate				Multivariate		
	N	%	DFS		HR (95% CI)	p value		N	%	DFS		HR (95% CI)	p value	
			mDFS (Range, Month)	HR (95% CI)	p value					mDFS (Range, Month)	HR (95% CI)	p value		
Adjuvant endocrine therapy														
TAM/TOR	41	67.2	52.0(9.0–197.0)	1.00		1.00		23	62.2	41.5(4.0–148.0)	1.00		1.00	
AI	14	23.0	53.0(16.0–177.0)	0.92(0.49–1.73)	0.792	0.52(0.13–2.06)	0.354	10	27.0	28.5(7.0–131.0)	1.30(0.61–2.78)	0.501	1.53(0.43–5.42)	0.508
TAM plus AI	3	4.9	100.5(63.0–138.0)	0.50(0.12–2.10)	0.343	1.00(0.12–8.68)	0.998	3	8.1	50.0(32.0–72.0)	0.95(0.28–3.21)	0.932	1.05(0.09–12.54)	0.967
Others	3	4.9	153.0(35.0–197.0)	0.32(0.10–1.09)	0.069	NA	NA	1	2.7	NA	0.36(0.05–2.78)	0.330	NA	NA
Adjuvant chemotherapy														
PTX	9	12.5	60.0(23.0–122.0)	1.00		1.00		12	25.0	39.0(23.0–123.0)	1.00		1.00	
Anthracycline	23	31.9	55.5(18.0–197.0)	0.77(0.35–1.69)	0.507	3.29(0.78–13.89)	0.106	7	14.6	34.0(5.0–66.0)	1.72(0.66–4.48)	0.265	1.38(0.18–10.32)	0.754
PTX plus anthracycline	36	50.0	48.0(9.0–177.0)	1.03(0.49–2.18)	0.932	0.78(0.24–2.59)	0.688	25	52.1	24.0(9.0–131.0)	1.43(0.71–2.88)	0.323	1.59(0.35–7.22)	0.550
Others	4	5.6	44.0(30.0–84.0)	1.30(0.40–4.25)	0.661	2.26(0.19–27.58)	0.523	4	8.3	72.0(9.0–117.0)	0.73(0.20–2.63)	0.629	0.47(0.02–12.22)	0.650

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; mDFS, median disease free survival; HR, hazard ratio; CI, confidence interval; NA, unknow; AI, aromatase inhibitor; TAM, tamoxifen; TOR, toremifene; PTX, paclitaxel.

TP53 Status Was Associated with Adjuvant Endocrine Therapy Response

A total of 96 patients who received adjuvant endocrine therapy were selected to evaluate the relationship between *TP53* mutation status and the response to endocrine therapy. As shown in Table 3, we found that 84.7% (50/59) of patients accepted adjuvant chemotherapy in *TP53* wild-type group, whereas 78.4% (29/37) of patients accepted adjuvant chemotherapy treatment in *TP53* mutant patients. There was no significant difference between *TP53* status and adjuvant chemotherapy (P=0.467). As well known, ESR1 mutations are associated with acquired endocrine resistance in breast cancer so that we took ESR1 mutation rate into consideration in Table 3, but there were no significant differences in ESR1 mutation rate (p=0.558) between the two groups.

To further explore the relationship between *TP53* status and treatment response, we classified patients into the adjuvant endocrine therapy-resistant group and the adjuvant endocrine therapy sensitive group. Interestingly, we found that in the adjuvant endocrine therapy sensitive group, patients with *TP53* mutations had a significantly shorter DFS than *TP53* wild-type patients (69.0 months vs 108.0 months, respectively, hazard ratio=3.22, 95% CI=0.70–14.77, P=0.008) (Figure 6B). No significant DFS differences between *TP53*-mutated and *TP53* wild-type patients were seen in the endocrine therapy-resistant group (34.0 months vs 40.0 months, respectively, P=0.903) (Figure 6A).

Discussion

In our study, we used NGS to detect *TP53* mutations in the cfDNA, which might affect tumor temporal and spatial heterogeneity, of 187 Chinese MBC patients. Our results indicated that *TP53* mutations could be used as a prognostic marker for worse outcome in MBC and for the response of adjuvant endocrine therapy.

We established genomic profiles of patients which revealed a *TP53* mutation frequency of 42.2%, similar to that seen in the Guangdong Provincial People's Hospital cohort (45.0%) but higher than in the TCGA breast cancer cohort (30.0%).[26] Another recent study on cfDNA molecular profiling in Chinese patients with MBC reported a *TP53* mutation rate of 64.1% compared with 52% in Caucasian patients.[27,28] These discrepancies could reflect differences between patient ethnicities, such as in the median age of breast cancer patients with *TP53* mutations in our study of 48 years compared with 55.2 years in Caucasians.[29]

Table 3 Clinical Characteristics of Patients Receiving Adjuvant Endocrine Therapy (n=96)

Characteristics	*TP53* Status		p value
	Wild-Type (n=59)	Mutated (n=37)	
Age at diagnosis (years)			
≤50	38(64.4%)	26(70.3%)	0.553
>50	21(35.6%)	11(29.7%)	
Stage at diagnosis			
I~II	36(61.0%)	19(51.4%)	0.273
III~IV	16(27.1%)	14(37.8%)	
Unknow	7(11.9%)	4(10.8%)	
Grade			
I–II	30(50.8%)	14(37.8%)	0.257
III	7(11.9%)	1(2.7%)	
Unknow	22(37.3%)	22(59.5%)	
Ki67			
1~20%	24(40.7%)	15(40.5%)	0.820
>20%	26(44.1%)	18(48.7%)	
Unknow	9(15.2%)	4(10.8%)	
Lymph node status			
Positive	37(62.7%)	22(59.5%)	0.928
Negative	21(35.6%)	13(35.1%)	
Unknow	1(1.7%)	2(5.4%)	
Disease involvement			
Visceral	38(64.4%)	23(62.2%)	0.824
Non-visceral	21(35.6%)	14(37.8%)	
Adjuvant endocrine therapy			
Tamoxifen/toremifene	41(69.5%)	23(62.2%)	0.238
Aromatase inhibitor	14(23.7%)	10(27.0%)	
Tamoxifen+ aromatase inhibitor	3(5.1%)	3(8.1%)	
Others	1(1.7%)	1(2.7%)	
Adjuvant chemotherapy			
Yes	50(84.7%)	29(78.4%)	0.467
No	8(13.6%)	7(18.9%)	
Unknow	1(1.7%)	1(2.7%)	
ESR1 mutation			
With *ESR1* mutation	7(11.9%)	3(8.1%)	0.558
Without *ESR1* mutation	52(88.1%)	34(91.9%)	

The p53 pathway was previously shown to rank top in the basal-like breast cancer subtype, but not in the HER2-enriched type; therefore, *TP53* mutations were not associated with poor prognosis in the HER2-enriched group.[6] In support of this, our data indicated that the *TP53* mutation status was an independent predictive factor of survival especially in HR+/HER2– and TNBC cohorts, but not in the HER2-positive cohort.

Several studies have shown that the DBD is the most frequently mutated *TP53* region in breast cancer. In line with this, codons 175, 220, and 248 located within the DBD were the site of many *TP53* mutations in our study, of which most were missense mutations. DBD mutations were previously reported to have prognostic value,[30,31] while non-missense mutations were associated with a worse outcome in MBC.[32] A recent study showed that missense mutation in the DNA-binding domain had dominant-negative effects (DNE).[33] There was no difference in survival between patients with dominant-negative p53 mutant tumors and those with *TP53* mutations that are predicted to be non-dominant negative.[34,35] In our study, *TP53* missense mutations in the DBD were associated with improved survival. Further analysis showed that patients with *TP53* mutations in the non-DBD had a significantly shorter DFS than those in the *TP53* non-mutation cohort.[36] In order to investigate the prognostic value of p53 protein further, we divided them into *TP53* wild-type group; protein stable mutations group and protein non-stable mutations group. In our study, patients with protein non-stable mutations had significantly shorter DFS and OS than *TP53* wild-type patients. Moreover, protein non-stable mutations included all truncating and frame-altering mutations, and mutations in the tetramerization domain so that mutations in TD had a worse clinical outcome. The reasons were that mutations in TD could either abolish or reduce binding of p53 protein to DNA and transcriptional activation, and *TP53* mutation in TD domain had dominant-negative effects (DNE) that inactivate *TP53* wild type in some cases.[37] Other researchers also found mutations in TD domain were associated with cancer-associated development.[38] Not all missense mutations cause protein accumulation, while non-missense mutations are true loss-of-function mutations. Thus, missense mutations have generally been associated with higher protein expression compared with non-missense mutations.[16]

Some clinical trials showed us *TP53* might be the potential to be a therapeutic biomarker. Studies on the role of *TP53* mutation in breast cancer response to chemotherapy are conflicting.[39–42] Data on the association between *TP53* mutations and endocrine therapy response were also controversial.[43–45] When it came to the association between hormone therapy and chemotherapy, some researchers found that adding hormone therapy to chemotherapy could improve the survival for *TP53* wild-type patients not for

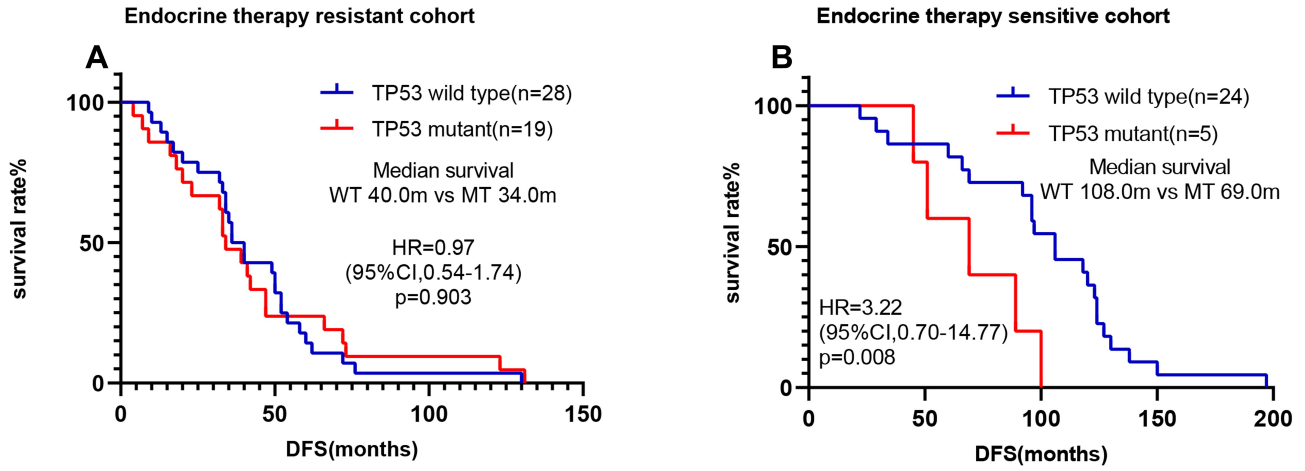

Figure 6 Survival analyses by Kaplan–Meier according to TP53 status in MBC receiving adjuvant endocrine therapy. (**A**) There was no significant difference in TP53 status in the endocrine therapy-resistant cohort. (**B**) TP53 wild-type patients had a significantly better clinical outcome than TP53-mutated patients in the endocrine therapy sensitive cohort.

TP53 mutation patients.[46,47] While in our research, 84.7% of patients in *TP53* wild-type group and 78.4% patients in *TP53* mutant group all accepted adjuvant chemotherapy and endocrine therapy treatment in Table 3, and the distribution of patients with adjuvant chemotherapy was balanced in two groups, which did not exert an influence on the analysis of endocrine therapy and *TP53* status. In our study, we also found *TP53* mutations were associated with endocrine resistance. *TP53*-mutated patients had a shorter DFS than *TP53* wild-type patients in the adjuvant endocrine therapy sensitive group. Previously, increased expression of estrogen-related receptor (ERR)α was associated with increased levels of p53 in ERα-positive cases. ERα and ERRα share only 33% homology in their ligand-binding domains, resulting in the insensitivity of ERRα to tamoxifen.[48] Additionally, *TP53* wild-type tumors might be more responsive to endocrine therapy because this disrupts the ERα–p53 interaction and reactivates p53.[49]

The retrospective nature of our study resulted in a number of limitations. DFS might have influenced the survival analysis, which was retrospectively calculated. Additionally, we lacked matched primary and recurrence samples for analysis. Finally, we did not analyze p53 protein expression to verify our results.

Conclusion

In conclusion, *TP53* wild-type MBC patients showed better survival than *TP53*-mutated patients in HR+/HER2– and TNBC cohorts. Missense mutations in the DBD of p53 appeared to be an independent prognostic marker for short DFS, while *TP53* mutations were associated with endocrine resistance. This indicates that alternative therapies for HR-positive patients with *TP53* mutations should be considered. Large-scale prospective studies are needed to verify our findings.

Data Sharing Statement

We can provide the original data in this manuscript upon request.

Research Ethics and Consent

The written informed consent of this research had been provided by the patients, and this study was approved by the Medical Ethics Committee of Peking University Cancer Hospital & Institute (Approval No.2016KT47) according to the Declaration of Helsinki.

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